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Genetic Variation of Invasive Aquatic Plant *Elodea nuttallii* (Planch.) St. John (*Hydrocharitaceae*) in Japan

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Elodea nuttallii (Planch.) St. John is a submerged aquatic plant, which is native in temperate North America. It has invaded Japan from Hokkaido to Kyushu but the time and the course of the invasion are not clear. The RAPD analysis of this study showed that genetic variation within *E. nuttallii* in Japan for the first time. The result suggests multiple invasions of *E. nuttallii* to Japan in the past.

Key words: aquatic plant, *Elodea nuttallii*, genetic variation, invasive species, RAPD.

Elodea nuttallii (Planch.) St. John (*Hydrocharitaceae*) is a submerged aquatic plant, which is native in temperate North America (Cook and Urmi-König 1985). Currently it has invaded Europe (Cook and Urmi-König 1985, Cook 1996) and Japan (Kadono 1994). It had been introduced to Japan as an experimental material for research in plant physiology (Ohtaki and Ishido 1980). However, the time when it was introduced is not cleared since there are no records such as herbarium specimens (Kadono 1994). The first record of this plant in Japan is Lake Biwa from in 1961 (Ikusima and Kabaya 1965). After that, *E. nuttallii* has spread widely in Japan, from Hokkaido to Kyushu (Kadono 1994, Takita and Kadono 1997), and has outcompeted native species from their habitats in some areas (Kurita and Minemura 1985a, 1985b, Hoshi 1989). On the other hand, some studies showed that populations of *E. nuttallii* decline after exuberant growth in some areas (Momose 1986, Funakoshi 1989). Simpson (1984) studied the process of growth of *Elodea canadensis* in the British Isles and showed that it propagated for three to four years after invasions. Then, they became dominant in next three to ten years and declined eventually in the following seven to fifteen years. With regard to the cause of a series of decline after increase of *E. nuttalii*, Sculthorpe (1967) suggested two following hypotheses about this process: one is lack of genetic variability among the invaded population and another is limitation of nutrient in their habitats. At present, we cannot discuss these hypotheses, especially for the former one, as we have no genetic information of the species.

To assess genetic variation in wild plants, random amplified polymorphic DNA (RAPD) as well as allozyme analysis has been widely used (Maki and Horie 1999, Fisher et al. 2000, Ceplitis 2001, Tanaka et al. 2002). In general, it is difficult to detect many allozyme loci in wild plant species because plant tissues contain many inhibitors of enzyme activity (Wendel and Weeden

1989). In contrast, RAPD is able to detect ample genetic polymorphism by screening entire genomes using many primers with different DNA sequences (Edwards 1998).

Kadono et al. (1997) analyzed allozyme banding patterns using five enzymes in 26 separate *E. nuttallii* plants collected from 26 sites in Japan. They concluded that there is no variety in *E. nuttallii* plants in Japan because no variation of band patterns was observed. Because only male plants of *E. nuttallii* were found in Japan, it was considered that all plants had been derived from a clone of the first invaded population and propagated asexually (Kadono 1994). However, further studies are needed to conclude whether *E. nuttallii* growing in Japan is a sole clone or not. In this study, we applied RAPD analysis with higher resolution capability for *E. nuttallii* and investigated their genetic variation in Japan.

Materials and Methods

Each individual of *E. nuttallii* (Planch.) St. John from 11 sites was collected and used for study (Fig. 1). Leaf surface was cleaned up and then stored at –80ºC. Total DNA was extracted from deep-frozen leaves and purified as described by Tanaka et al. (2003). RAPD amplifications were performed using arbitrary primers according to Williams et al. (1990). The following cycling profile was used for all reactions: 2 min at 94ºC, 1 min at 36ºC, 2 min at 72ºC for 1 cycle; 1 min at 94ºC, 1 min at 36ºC, 2 min at 72ºC for 44 cycles. Sixty primers (OPAA-01-20, OPN-01-20, OPV-01-20) from Operon Technologies Inc. were used in this study. The

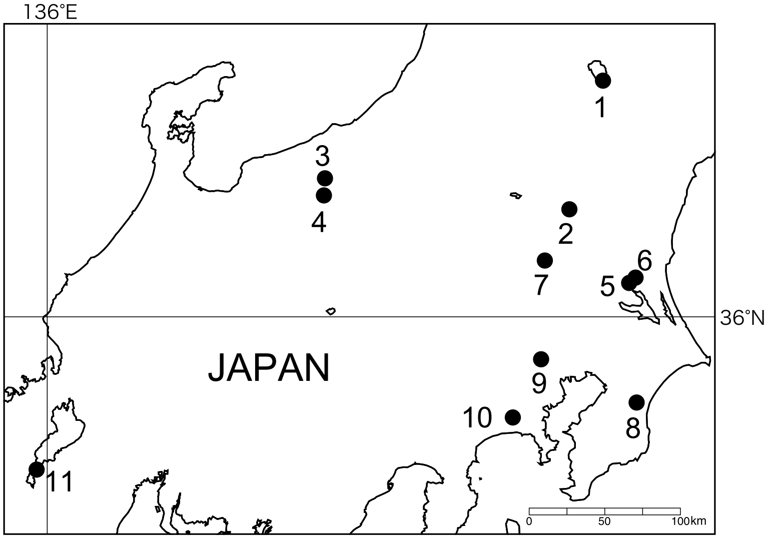

Fig. 1. Map of collection sites of *Elodea nuttallii* in Central Japan. Locality and accession numbers of voucher specimens are as follows. Vouchers are deposited in the Herbarium, National Museum of Nature and Science, Japan (TNS). 1. Lake Inawashiro–Inawashiro, Fukushima Pref. 2. Gogyo River–Haga, Tochigi Pref. (TNS 9540984), 3. Lake Nakatsuna–Ohmachi, Nagano Pref. (TNS 9540986). 4. Lake Kizaki–Ômachi, Nagano Pref. (TNS 9540985). 5. Amano River upstream–Yahata-shita, Chiyoda, Ibaraki Pref. 6. Amano River downstream–Nakagoyatsu, Chiyoda, Ibaraki Pref. (TNS 9540987). 7. Kasai Water Park–Kawamata, Hanyû, Saitama Pref. (TNS 9540988). 8. Ojaga Pond–Tanaka, Tôgane, Chiba Pref. (TNS 9540989). 9. Ya River–Yaho, Kunitachi, Tokyo Pref. 10. Zenmyoji River–Shimokawairi, Atsugi, Kanagawa Pref. 11. Lake Biwa–Yamanoshita Bay, Ôtsu, Shiga Pref. (TNS 9540990).

products were analyzed by electrophoresis on 1.3% agarose gels and visualized with ethidium bromide staining. Each DNA sample was applied for replicated running amplifications to confirm the banding pattern. UPGMA clustering was calculated based on the inconsistency rate between each plant.

Results and Discussion

Of sixty primers used in the preliminary analysis, nine RAPD primers produced a total of 16 polymorphic, resolvable and stable bands under the described PCR condition (Table 1). A gel photo of the banding pattern from OPN-14 marker is shown in Fig. 2. The band has a size ranging from 550 to 2,500 bp. Although variation was not recognized between sites 5 and 6, the distinct bands were seen at the other sites (Table 2). After transferring the band patterns into a 0/1 matrix, the data set was analyzed by Unweighted Pair-Group Method with Arithmetic mean (UPGMA). A UPGMA dendrogram based on inconsistent degree among all site was given (Fig. 3). In this dendrogram, plants from the upstream and downstream sites of the Amano River (sites 5 and 6) formed a cluster, but neighboring collection sites in the different water areas such as “Lake Nakatsuna (site 3) and Lake Kizaki (site 4)” and “Gogyo River (site 2) and Kasai Water Park (site 7)” did not form a single cluster.

In this study, we can show that *E. nuttallii* has genetic variation in Japan. The result indicates that several plants of *E. nuttallii* had invaded and spread over Japan because all *E. nuttallii* in Japan are male and expected to propagate asexually. Two hypotheses are possible as the process for invasion of the plant. First, several plants invaded Japan just one time. Second, the invasion might occur more than once. If *E. nuttallii* invaded Lake Biwa at first, and then spread throughout Japan from the lake, as Ikusima (1980) stated, this population might have genetic variation from the beginning.

Our result obtained here is not consistent with that from allozyme analysis performed by Kadono et al. (1997), since they did not find any differences in 26 independent *E. nuttallii* plants. The cause of this inconsistency is considered to be due to the difference of sensitivity for detection of variation between RAPD and allozyme analyses. In general, the detection capability of RAPD

Table 1. List of primers, sequences and the approximate lengths of polymorphic markers observed in *Elodea nuttallii*

Primer	Sequence (5´–3´)	Marker bands (bp)
OPAA-9	AGATGGGCAG	550
OPAA-10	TGGTCGGGTG	1050
OPAA-18	TGGTCCAGCC	1350
OPN-2	CTCACGTTGG	750
OPN-8	ACCTCAGCTC	1950
OPN-11	TCGCCGCAAA	850, 950, 1150, 1600, 1700
OPN-14	TCGTGCGGGT	950, 1000, 1400
OPV-10	GGACCTGCTG	1400
OPV-12	ACCCCCCACT	2300, 2500

Table 2. Polymorphic markers observed in this study

Markers	Collection sites										
	1	2	3	4	5	6	7	8	9	10	11
OPAA-9-550	–	–	–	–	–	–	–	+	–	–	–
OPAA-10-1050	–	+	–	–	–	–	–	–	–	–	–
OPAA-18-1350	–	–	–	–	–	–	–	–	–	–	+
OPN-2-750	–	–	–	–	–	–	–	–	–	–	+
OPN-8-1950	–	–	–	–	–	–	–	–	–	–	+
OPN-11-850	–	+	–	–	–	–	–	–	–	–	–
OPN-11-950	–	–	–	+	–	–	–	–	–	–	–
OPN-11-1150	–	+	–	–	–	–	–	–	–	–	–
OPN-11-1600	+	–	–	–	–	–	–	–	–	–	–
OPN-11-1700	–	–	–	–	–	–	–	–	–	–	+
OPN-14-950	–	–	–	–	+	+	–	–	–	–	–
OPN-14-1000	–	–	–	+	–	–	–	–	–	–	–
OPN-14-1400	+	–	–	–	–	–	–	–	–	–	–
OPV-10-1400	–	–	+	–	–	–	–	+	–	–	–
OPV-12-2300	–	–	–	–	–	–	–	–	+	–	–
OPV-12-2500	–	–	–	–	–	–	+	–	–	–	–

+ or – show presence or absence of marker bands.

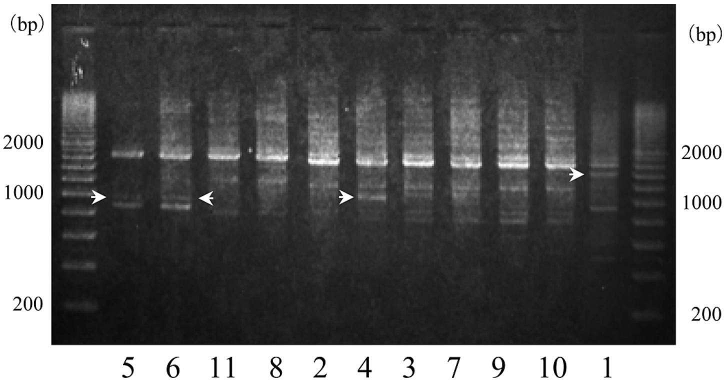

Fig. 2. RAPD banding patterns with the OPN-14 primer. Lane numbers show collection site numbers of *Elodea nuttallii* in this study. Lanes at both ends contain 200–2000 bp DNA ladder. Arrows indicate RAPD markers used for this study.

analysis is higher than that of allozyme analysis. Furthermore, our results, that genetic variation is observed in Japanese *E. nuttallii*, do not support the previous hypothesis that such a series of decline after increase as stated above is caused by the lack of genetic variability. However, it cannot be completely rejected without detection of genetic variation within each population. The pattern of a series of population decline after increase, and genetic structure in each population should be investigated in detail. The hypothesis that nutrient deficiency causes declines also requires verification.

Correlation between geographical distribution and genetic identity was not found in this study. Actually, we have found the genetic variation of *E. nuttalii* within Lake Biwa using AFLP analysis (data not shown). Population genetic analysis of more sites using more sensitive markers such as AFLP may reveal the pattern of dispersal in Japan.

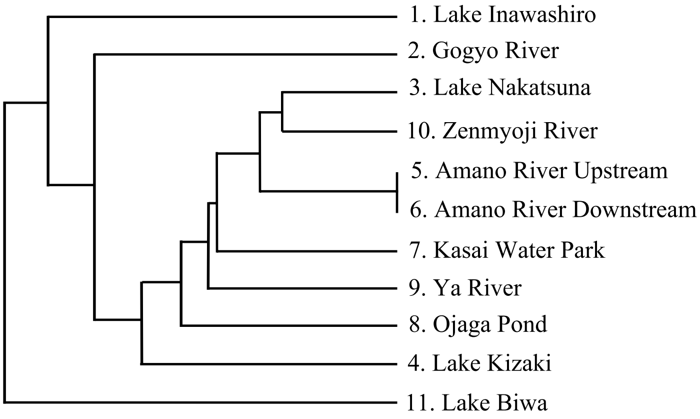

Fig. 3. UPGMA dendrogram based on the degree of inconsistency among all sites of *Elodea nuttallii* in Japan.

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田中法生[a]，冨田千賀子[b]，矢部　徹[b]，玉置雅紀[b]：**侵入水生植物コカナダモの日本における遺伝的変異**

コカナダモ *Elodea nuttallii* (Planch.) St. John は北アメリカの温帯域に原産する沈水植物であり，日本へは北海道から九州まで帰化しているが，侵入の時期や経路は明らかでない．従来，アロザイムパターンに全く変異が見られないことから国内のコカナダモは最初に侵入した単一クローンに由来すると考えられてきた．しかし，本研究のRAPD解析により，国内のコカナダモに遺伝的変異が見られることが初めて明らかになった．この結果から，複数の個体が過去に国内へ侵入したことが示された．

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